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TITLE: Phase I Trial of Anti-PSMA Designer T Cells in Advanced Prostate Cancer

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14. ABSTRACT

Anti-PSMA designer T cells are autologous T cells that are gene-modified to express chimeric immunoglobulin-T cell receptors (IgTCR) that recognize the prostate specific membrane antigen. We will conduct a Phase I dose-escalation trial with infusion of designer T cells into prostate cancer patients after non-myeloablative (NMA) conditioning. This procedure will allow for the stable engraftment and persistence of the infused cells for improved therapeutic effect. The first year efforts have been in preparation of a clinical-grade vector producer cell (VPC) line, production and testing of clinical vector supernatants, and in harmonizing IRB-recommended protocol modifications and completing administrative features in advance of the trial. In year 2, the trial is planned to begin enrolling patients.

15. SUBJECT TERMS
Designer T cells, gene therapy, prostate cancer, non-myeloablative conditioning, phase I trial

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ANNUAL REPORT

Introduction

The study is a clinical trial of anti-PSMA designer T cells in prostate cancer. The first year under the study has been focused on two major tasks: 1. finalizing vector cloning and selection, and vector production in collaboration with the National Gene Vector Lab (NGVL); and 2. completing administrative tasks in preparation for the clinical trial.

Body

Tasks from Statement of Work.

Task #1. Vector production/delivery

a. Cloning optimal vector producer cell (VPC)

Testing for the best MFG-S3D8V5 retroviral supernatant from PG13 transduced clones produced at the National Gene Vector Laboratory (NGVL) for the purpose of preparing our prostate gene therapy GMP supernatant. We provided purified plasmid DNA for the MFG vector to the NGVL from which they would create a new VPC under GMP conditions. This work to produce the VPC was done at the NGVL. In turn, the BDL conducts the tests of the titers to determine the best clones.

To find the best cells to use for this screening, we tested three cell types to determine which gives the best transduction rate. Jurkats (a human CD4 T cell lymphoblast cell line), 293 (human embryonic kidney cell line), and PBMCs (peripheral blood mononuclear cells, which is our source for fresh T cells) from buffy coats were tested. These cells were transduced two times with either IgTCR or Tandem vectors (two other test vectors in the lab). The best cell type of the three was the 293 cells which gave 69% transduction for the IgTCR and 44% for Tandem post 48-hr gene expression. The Jurkats gave 5% IgTCR and 7% Tandem, and the PBMCs only 0.6% IgTCR and 0.45% Tandem. A graphical analysis showed that there was a constant ratio between 293 cells and the normal activated T cells, but that titer was more sensitive with the 293 cells. Therefore, we did initial screening with the 293 cells and then confirmed relative titers in normal activated T cells, the cells that would be used for the clinical trial.

Testing 1st (38 clones) and 2nd (21 clones) batches of clones; retesting selected clones (12); final testing/selecting the best clone. The supernatants (1:3 dilutions) from 59 clones from NGVL were used to transduce activated T cells twice. Facs analyses were done; the 3 best clones (clones 1, 6 and 10) were retested in both 293 and activated T cells. Gene transfer rates for the 293 and the T cells after 3-day post gene expression were compared for the three clones. Clone 6 gave 66.1% in the 293 cells and 11.2% in the T cells compared with 55.3% (clone 1) and 49.2% (clone 10) in 293 and 10.2% (clone 1) and 6.8% (clone 10) in activated T cells. Clone 6 was selected as the best for manufacturing the GMP-grade supernatant for prostate cancer clinical trials.

Associated studies to improve gene therapy procedures:

1. As an adjunct study to support the trial, we attempted to validate a replication competent retrovirus (RCR) Serologic Assay using anti-CD46 antibody for detecting GALV envelope protein. The GALV envelope is reported to cross-react with CD46. This assay is based on antibody detection in the serum of patients. If the anti-viral antibody is detected by GALV-positive cells, that means the patient has retrovirus growing inside his body.

Serial dilutions of the antibody which putatively recognizes the retroviral envelope (GALV) were made over a range of 10 to 0.01 ug/ml sensitivity with NIH3T3 cells expressing GALV env. The antibody was not detected in this assay. It was concluded after two tests that this antibody does not work in this application as a positive control with which to validate the test. Therefore, it does not meet the FDA criterion for a controlled assay. Accordingly, the more expensive and prolonged culture assay for RCR will be continued for this study.

2. Test to determine if protamine sulfate (a) harms cells or lowers the growth potential of Jurkats and (b) somehow affects the background staining in Jurkats when doing FACS. An assay using 0, 5, and 10 ug/ml protamine sulfate in growth medium with Jurkats was tested. It was shown that viability and growth were not adversely affected, and background staining had not increased when compared with the lower amount or no protamine sulfate. (did it increase infection rate?)

3. We also examined more efficient mans for transduction to bring up the fraction of modified cells in the designer T cell preps. In doing a titer/dilution assay, we discovered an unexpected result with increased titer with increasing dilution. We determined infectious titer of dilutions of retroviral supernatant: 1, 1:3, 1:9, and 1:27 in Jurkats and 293 cells transduced with the best viral supernatant harvests from PG13/Tandem sorts 0-3. PG13/Tandem is a heterologous vector used for control tests. Prior to experiment, PG13/Tandem cells were sorted three times giving sorts 0-3, the PG13/ Tandem Sorts 0-3 were grown in culture until confluent. Supernatants were collected for 4 days from each of the PG13/tandem sorts 0-3, giving 4 harvests per sort; each harvest was used to transduce 293T cells once, then the transduced cells were stained for FACS analysis. The best harvest (supernatant) was chosen for each sort 0-3

Dilutions of supernatants were used to transduce 293 cells twice, then the cells were analyzed by FACS to determine % transduction. The infectious titer/ml supernatant (s/n) = initial number of cells x gene transfer rate (% transduction) x dilution factor. Plots of titer/ml vs dilution s/n; percent transduction vs dilution s/n; and mean fluorescence vs dilution s/n were made.

Results: (1) The infectious titer/ml s/n showed an increase with increasing dilutions of s/n (2) the gene transfer rate and the fluorescence intensity for all sorts was highest at the 1:3 dilution, sort 3 was slightly higher for the 1:9 dilution. This result may reflect an increase in nutrition for the cells thereby enhancing their infectivity. We may consider diluting the s/n 1:3 in future experiments.

This test was repeated again in January with similar results: in 3 out of four, the 1:3 dilution gave the highest gene transfer rate.

b. Production of clinical quantities of vector

The selected VPC clone (#6) was indicated to NGVL. The cells have been expanded at NGVL and the master cell bank (MCB) has been completed with safety testing is underway. At the time of this report, the viral supernatant production is pending (K Cornetta, MD, 7/14/06). The vector production is anticipated to be completed in August 06. Safety testing for supernatant requires 3 months. The vector is anticipated to be available for patient use in 11/06.

Task #2. Study preparations

a. Laboratory standardizations

The detection of designer T cells in the tumor will be by staining with the anti-V5 antibody that recognizes the V5 epitope that was inserted into the IgTCR extracellular portion. This will be done with prostate cancer tissue that has been injected with a dilute solution of designer T cells and then processed as fixed or frozen tissue with immunohistochemistry (IHC). Plans have been made with the Pathologist (M Stancu, MD) and the lab protocol manager (Q Ma, PhD) and the clinical fellow on the study (P Somasundar, MD) to complete this test. This should be completed in 8/06.

b. Staff training/orientation

Robin Davies, RN, research nurse, created Case Report Forms (CRF) for the Study that were submitted with the original application. These forms have been updated during the last year. A further draft version of the CRF has been prepared by Ms. Davies and Jinnie Shin, BA, data manager, so that more specific information can be collected. This is the primary training document for the medical staff who will be administering the T cells and managing the patients. The CRF will be finalized in 8/06. Katarzina Szulc was hired as Data Manager in 6/06 to replace J Shin and will continue the data manager's part of this effort under Ms. Davies's direction.

Task #3. Clinical protocol implementation

Principal Investigator Richard P Junghans, PhD, MD and Nurse Coordinator Robin Davies, RN attended the US Army Grants Award Meeting on May 4-5, 2005, where the outline of interactions between the grantees and the DOD was presented, including the compliance with the human subjects review of the DOD. This DOD meeting is used to mark the beginning of the past year's protocol activities. The clinical protocol (and consent) has undergone a number of revisions over the year to harmonize requirements between the DOD and RWMC IRBs. The history prior to the award is included to provide the full context of the activities undertaken in the period under the grant.

This study was initially submitted to the Roger Williams Hospital Human Research and Review Committee (IRB) on November 22, 2004. It was reviewed at a Full Committee meeting on December 21, 2004. Modifications were requested to the Protocol and Consent Form versions December 9, 2004. These modifications included a request to address the rationale for doses of chemotherapy used in the study. The response to these requests was submitted on December 28, 2004. The IRB then requested further information and changes in their second response dated January 14, 2005.

A second response to the IRB by the Principal Investigator was submitted on February 7, 2005. This included Protocol and Consent Form versions February 7, 2005. These versions were approved in a letter dated March 30, 2005. The approval was for the three month period March 30, 2005 through June 2005.

On May 12, 2005 the three-month report was submitted to the IRB with February 7, 2005 versions of the Protocol and Consent Form. This was approved on May 26, 2005.

Amendment #1 was submitted to the IRB on September 15, 2005 which included Cytoxan dose changes. The Annual review was then submitted on October 21, 2005. These were both reviewed on November 8, 2005. Requests for modifications were then made by the IRB. A response by the Principal Investigator was submitted on January 12, 2006. This response created Amendment #2 to the Protocol. These changes were accepted by the IRB and signed on January 23, 2006 to approve versions January 12, 2006 Consent Form and February 7, 2005 of the Protocol.

During this period of time the Principal Investigator had submitted an application for funding to the US Army. A working version of the Protocol and Consent form were submitted in the form of Protocol and Consent Form versions March 28, 2005. This version was reviewed at the US Army Human Subject Research and review Board (HSRRB) on September 14, 2005. As a result of this review, recommendations for modifications to both the Consent Form and Protocol were made. This request included 14 changes to the Consent Form, 14 changes to the Protocol and 11 additional items needing response and/or action. These changes were implemented and resulted in versions October 15, 2005 of the Protocol and Consent Form.

On May 17, 2006 a request for final Roger Williams Hospital IRB approval of the Protocol and Consent Form was received from HSRRB. Since the Roger Williams Hospital had previously approved only RWH versions February 7, 2005 of the Protocol and version January 12, 2006 of the Consent Form, it was decided to submit the now HSRRB reviewed/approved (October 15, 2005 versions) of the Protocol and Consent Form to the RWH IRB. These were submitted in the form of Protocol (Amendment #3) version February 14, 2006 and Consent Form version February 14, 2006. A letter conditionally approving both Protocol and Consent Form was received on April 3, 2006, from RWH IRB. Modifications were requested to the Consent only resulting in version June 15, 2006. The final approval is pending.

Enrollment is expected to commence upon receipt and testing of the vector. Vector will be supplied from the National Gene Vector Laboratory (NGVL). There has been a queue of other

vectors ahead of ours. The NGVL recently informed us that production is due to begin shortly with anticipated delivery time of Fall, 2006. The patient enrollments will begin shortly after.

Task #4. Data management

The Case Report Form (CRF) has been created in draft form. A Study calendar was created for Adverse Event reports and Annual reports, with contact information for all involved agencies.

Key Research Accomplishments

None to report at this time.

Reportable Outcomes

None to report at this time.

Conclusions

The study has moved forward in the vector preparations and in the study administration. It is anticipated that enrollments can begin in Fall of 2006.

References

None.

Appendices

None.